may be used to detect a variety of tricyclic antidepressant drugs, allowing any of the drugs to be tested using a single test.

Screening patients for drugs of abuse in the urine may be indicated to help differentiate symptoms, or to insure that a patient is substance-free before undergoing medical procedures. Drug screening of pregnant women with a history of drug abuse may be useful as an educational tool and help guide treatment of the newborn. In addition, some employers require a drug screen as part of an employment or pre-employment physical. Nearly all workers in some occupations, such as law enforcement and transportation, are subject to periodic, random, and post-incident drug screening. The chemical sensor array may be used to detect a variety of drugs of abuse in a quick and easy manner. Typically, a variety of different tests must be used to test for each class of drug. By incorporating multiple particles into a single sensor array, some or all of the most commonly used drugs of abuse may be determined in a single step.

Urine screening tests for drugs of abuse detect general classes of compounds, such as amphetamines, barbiturates, benzodiazepines, or opiates. Drug screening also includes testing for cocaine, marijuana, and phencyclidine (PCP). The screening test for cocaine detects benzoyl ecgonine, the major metabolite of cocaine. The marijuana test detects D-9-tetrahydrocannabinol, a principle product of marijuana smoke. One problem of the screening test is that the test, in some instances, may not be able to distinguish between illicit drugs and prescription or over-the-counter compounds of the same class. A patient taking codeine and another taking heroin would both have a positive screening test for opiates. Some over-the-counter medications can cause a positive drug screen in a person who has not taken any illegal or prescription drugs. For instance, over-the-counter sympathomimetic amines such as pseudoephedrine and phenylpropanolamine may cause a false-positive screen for amphetamines. Eating food containing poppyseeds may result in a positive urine screening test for opiates, since poppyseeds contain naturally-occurring opiates. However, confirmation testing will distinguish between positive opiate tests resulting from poppyseed ingestion and those resulting from heroin or other opiates, because different metabolic breakdown products are present. Monoacetylmorphine (also

called 6-monoacetylmorphine or 6-MAM) is a heroin metabolite. The presence of this metabolite is conclusive evidence that heroin was ingested.

Most of these problems of false positive results may be avoided through the use of a sensor array. The sensor array may include a variety of particles, each specific for a particular drug. Some of the particles may be specific designed to interact with the drug of abuse, for example amphetamine. Other particles may be designed to interact with an over-the-counter drug such as pseudoephedrine. The use of a variety of particles may allow a more accurate or complicated analysis to be performed through the use of a pattern recognition system. Even though many of the drugs may react with one or more particles, the pattern and intensity of the signals produced by the particles in the sensor array may be used to determine the identity of the drugs present in the patient. The most commonly used test method for screening urine for drugs of abuse is immunoassay. A number of single use devices incorporating immunoassays and designed to be used outside of the traditional laboratory are currently available.

Hyperglycemia can be diagnosed only after ruling out spurious influences, especially drugs, including caffeine, corticosteroids, indomethacin, oral contraceptives, lithium, phenytoin, furosemide, thiazides, etc. Thus, a sensor array may be used to expedite diagnosis of hyperglycemia by determining the presence of drugs that may cause false positives.

In another embodiment, a sensor array may be used to asses the presence of toxins in a person or animal's system. In general, toxins may be any substance that could be ingested that would be detrimental to one's health. For animals, a few examples of toxins include lead, organic phosphates, chlorinated hydrocarbons, petroleum distillates, alkaloids (present in many types of poisonous plants), ethylene glycol, etc. People may ingest a variety of these compounds, along with a number of different types of drugs, either over-the counter, prescription, or illegal. In many instances the patient, either animal or human, may exhibit symptoms which indicate the presence of a poison, however, the diagnosis of the particular poison ingested by the person may be difficult. This may be particularly difficult for animals or children, since the owner may not

know what the animal/child has eaten. For people, if the poisoning is severe, the person may be unconscious and unable to tell the physician the cause of the poisoning.

The use of a sensor array, may allow a medical expert to accurately and quickly assess the types of toxins present in a patient. A single sensor array may hold particles that are reactive to a wide variety of toxins. A single analysis of a sample of the patients bodily fluids (e.g., blood) may allow the medical expert to determine the identity of the poison. Once identified, the proper treatment may be used to help the patient.

A sensor array may also be used for soil testing. As with the grain testing, the testing of soil samples may require an extraction of the soil samples by a suitable solvent. For metals and other inorganic salts, the solvent used may be either water or dilute aqueous acid solutions. The soil may also be extracted with organic solvents to extract any organic compounds that are present in the soil sample. These solution containing the extractable material may then be analyzed using a sensor array. The sensor array may include particles that are specific for a variety of soil contaminates such as paints, lead, phosphates, pesticides, petroleum products, industrial fallout, heavy metals, etc. The use of a sensor array may allow one or more of these materials to be simultaneously analyzed in a soil sample.

EXAMPLES

In the below recited table are examples of analytes that have been detected using the sensor array system described herein. In the Receptor/Enzyme column are listed examples of receptors that may be used for the corresponding analyte. These receptors are covalently bound to a polymeric resin, using methods described herein.

Analyte	Type	Receptor/Enzyme
Sodium, Potassium	Small Molecule (Electrolyte)	Crown ethers, cryptands, chromoionophores such as Chromolyte® (from Bayer), Enzymes such as β-galactosidase, or other metalloenzymes.
Bicarbonate	Small Molecule (Electrolyte)	Enzymes such as Carbonic anhydrase
Calcium	Small Molecule (Electrolyte)	Complexometric dyes such as Arsenazo III, Xylenol Orange, Alizaren Complexone
Magnesium	Small Molecule (Electrolyte)	Complexometric dyes such as Calmagite, Magon
Chloride	Small Molecule (Electrolyte)	Enzymes and/or small molecule detectors such as Amylase, Phenyl mercury compounds, mercuric thiocynanates, diphenylcarbazones
Oxygen	Small Molecule (Metabolite)	Oxygen complexing molecules such as porphyins, synthetic hemeglobins, Ruthenium trisbipyridine
Carbon dioxide	Small Molecule (Metabolite)	Enzymes such as Carbonic anhydrase
pH	Small Molecule (Electrolyte)	PH indicator dyes such as Hydroxynitrophenylacetic acid, Congo Red, Brilliant Yellow, Carboxyphenolphthalein
Creatinine	Small Molecule (Metabolite)	Enzymes such as Creatinine deiminase or small molecule detectors such as picrate
Urea	Small Molecule (Metabolite)	Enzymes such as Urease
Glucose	Small Molecule (Metabolite)	Enzymes such as Gluocose oxidase/Peroxidase
Hepatitis B	Virus	Antigen/antiboby pairs such as Hepatitis B surface antigen
Feline Leukemia	Virus	Antigen/antiboby pairs such as FeLV antigen
Cytokines Interleukin 1 Interleukin 2 Interleukin 4 Interleukin 6 Interleukin 10 Gamma Interferon Tumor Necrosis Factor (TNF)	Small Molecule (Markers), Cellular signals	Small molecule markers and/or antigen/antibody pairs

Nucleic Acid Identification Methodology

In one embodiment, the chemical sensor array may be used for the determination of the sequence of nucleic acids. Generally, a receptor may be attached to a polymeric bead to form a particle. The receptor may have a specificity for a predetermined sequence of a nucleic acid. Examples of receptors include deoxyribonucleic acids (DNA) natural or synthetic (e.g., oligomeric DNA), ribonucleic acids (RNA) natural or synthetic, and enzymes. A number of methods may be used to analyze a nucleic acid to determine its sequence. The methods, summarized below, may be adapted for use in the previously described chemical sensor array to analyze a sample which includes a nucleic acid analyte.

In one embodiment, hybridization may be used to identify nucleic acids. This method relies on the purine-pyrimidine pairing properties of the nucleic acid complementary strands in the DNA-DNA, DNA-RNA and RNA-RNA duplexes. The two strands of DNA are paired by the establishment of hydrogen bonds between the adenine-thymine (A-T) bases and the guanine-cystosine (G-C) bases. Hydrogen bonds also form the adenine-uracil (A-U) base pairs in the DNA-RNA or RNA-RNA duplexes. Hybridization is highly sequence dependent. Sequences have the greatest affinity with each other where, for every purine in one sequence (nucleic acid) there exists a corresponding pyrimidine in the other nucleic acid and vice versa. The target fragment with the sequence of interest is hybridized, generally under highly stringent conditions that tolerate no mismatches. United States Patent 6,013,440 to Lipshutz, et al. describes hybridization in further detail and is incorporated by reference as if fully set forth herein.

Despite the high specificity of hybridization, there may be some mismatched nucleic acid strands. There are several ways to prevent mismatched strands from causing false positives. Ribonuclease enzymes may be used to dispose of mismatched nucleic acid pairs forming a RNA/DNA or RNA/RNA hybrid duplex. There are many types of ribonuclease enzymes that may be used for this purpose, including RNase A, RNase T1 and RNase T2. Ribonuclease enzymes specifically digest single stranded RNA. When RNA is annealed to form double stranded RNA or an RNA/DNA duplex, it may no longer be digested with these enzymes. When

a mismatch is present in the double stranded molecule, however, cleavage at the point of mismatch may occur. In one embodiment, a label may be attached to the RNA coupled to the particle. In the presence of a mismatch, cleavage may occur at the point of the mismatch. The cleavage may cause the labeled fragment to fall off the bead, causing a decrease in the signal detected from the bead. If the nucleic acid are perfectly complementary, then the fragment may remain uncleaved in the presence of the ribonuclease enzymes and the intensity of the signal produced by the particle may remain unchanged.

S1 Nuclease Cleavage may also be used to cleave mismatched pairs. S1 nuclease, an endonuclease specific for single-stranded nucleic acids, may recognize and cleave limited regions of mismatched base pairs in DNA:DNA or DNA:RNA duplexes. Normally, for S1 Nuclease to recognize and cleave a duplex a mismatch of at least about four consecutive base pairs is required. In a similar manner as described above, the cleavage of a labeled nucleic acid fragment may indicate the presence of a mismatched nucleic acid duplex.

T4 endonuclease VII (T4E7) and T7E1 are small proteins from bacteriophages that bind as homodimers and cleave aberrant DNA structures including Holliday Junctions. These molecules preferentially cleave mismatched duplexes. (Described in Youil R, Kemper B, Cotton RGH. Detection of 81 of 81 Known Mouse Beta-Globin Promoter Mutations With T4 Endonuclease-VII - The EMC Method. Genomics 1996;32:431-5, incorporated by reference as if fully set forth herein).

In another method, Chemical Cleavage of Mismatches (CCM) may be used. This technique relies upon the use of intercalation. Examples of intercalators include, but are not limited to, the chemicals hydroxylamine and osmium tetroxide to react with a mismatch in a DNA heteroduplex. Mismatched thymines are susceptible to modification by osmium tetroxide (or tetraethyl ammonium acetate and potassium permanganate) and mismatched cytosines can be modified by hydroxylamine. The modified bases are then cleaved by hot piperidine treatment. In a similar manner as described above, the cleavage of a labeled nucleic acid fragment may indicate the presence of a mismatched nucleic acid duplex.

In another embodiment, DNA-binding proteins may be used to identify nucleic acids. Most sequence-specific DNA-binding proteins bind to the DNA double helix by inserting an α-helix into the major groove (Pabo & Sauer 1992 Annu. Rev. Biochem. 61. 1053-1095; Harrison 1991 Nature (London) 353, 715-719; and Klug 1993 Gene 135, 83-92). United States Patent No. 5,869,241 to Edwards, et al. describes in detail methods for identifying proteins having the ability to bind defined nucleic acid sequences and is incorporated by reference as if fully set forth herein. In an embodiment, the DNA-binding proteins may be attached to a polymeric particle. The DNA-binding proteins may interact with the polymeric particle to produce a signal using a variety of the previously described signaling protocols.

Mispair Recognition Proteins, e.g., MutS, may also be used to detect mismatched base pairs in double-stranded DNA. There are several methods by which Mispair Recognition Proteins can be used. Mispair Recognition Proteins may bind to a mismatched base pair. Modified forms of a mismatch recognition protein may cleave a heteroduplex in the vicinity of a mismatched pair. A mismatch repair system dependent reaction, e.g., MutHLS, may be used for mismatch-provoked cleavage at one or more GATC sites. A mismatch repair system may be used in the formation of a mismatch-provoked gap in heteroduplex DNA. Mismatch-containing nucleotides may be labeled with a nucleotide analog, e.g., a biotinylated nucleotide. Molecules containing a base pair mismatch may be removed through the binding of the mismatch to the components of the mismatch repair system or by the binding of a complex of a mismatch and components of a mismatch repair system to other cellular proteins. Molecules containing mismatches may also be removed through the incorporation of biotin into such a molecule and subsequent removal by binding to avidin. The use of Mispair Recognition Proteins is described in detail in United States Patent 6,008,031 to Modrich, et al., which is incorporated by reference as if fully set forth herein. Hsu IC, Yang QP, Kahng MW, Xu JF. Detection of DNA point mutations with DNA mismatch repair enzymes. Carcinogenesis 1994;15:1657-62. 1, which is incorporated by reference as if fully set forth herein, describes the use of MutY in combination with thymine glycosylase for mismatch detection.

Yet another technique is Oligonucleotide Ligation Assay. In this method, the enzyme DNA ligase is used to join two oligonucleotides, annealed to a strand of DNA, that are exactly juxtaposed. A single base pair mismatch at the junction of the two oligonucleotides will prevent ligation. Ligation is scored by assaying for labels on the two oligonucleotides becoming present on a single molecule.

In another embodiment, an intercalating molecule may be used as a receptor. The combination of the intercalator with the polymeric bead may be used as a particle for a sensor array system. Intercalators typically react with duplex DNA by insertion into the duplex DNA. If the intercalator has a visible or ultraviolet absorbance or fluorescence, the wavelength or intensity of the intercalators signal may be altered when the intercalator is intercalated into duplex DNA. Examples of such intercalators include, but are not limited to, ethidium bromide, POTO, and Texas Red. Many intercalators exhibit some sequence selectivity. Thus, an intercalator bound to a polymeric resin may be used to analyzing DNA analytes for specific sequences. By using a variety of different intercalators in a single sensor array, the identity of the nucleic acid may be identified through a pattern recognition methodology.

The use of particles that are custom made for a variety of different nucleic acid testing schemes allows greater flexibility than the current commercially available nucleic acid devices. For example, the use of silicon chips in which the nucleic acid receptor is coupled directly to the chip may be less flexible since the size of the oligomeric receptor built onto the chip is limited to 25-30 base pairs. Methods for synthesizing oligomeric nucleic acids on a bead, however, may be used to couple oligomeric nucleic acids which include more than 100 base pairs.

Tests used to identify nucleic acids sometimes require that the amount of nucleic acid in the sample be increased. Techniques have been developed to amplify the chemical of interest. For example, it is possible to control which strand of a duplex nucleic acid is amplified by using unequal amounts of primer so that the primer for the undesired strand is effectively rate limiting during the amplification step. Methods of determining appropriate primer ratios and template

sense are well known to those of skill in the art (see, e.g., PCR Protocols: a Guide to Methods and Applications, Innis et al., eds. Academic Press, Inc. N.Y. 1990).

Polymerase Chain Reaction (PCR) is a widely used technique which enables a scientist to amplify DNA and RNA sequences at a specific region of a genome by more than a millionfold, provided that at least part of its nucleotide sequence is already known. The portions on both sides of the region to be amplified are used to create two synthetic DNA oligonucleotides, one complementary to each strand of the DNA double helix, which serve as primers for a series of synthetic reactions which are catalyzed by a DNA polymerase enzyme. Effective amplification may require up to 30 to 40 repetitive cycles of template nucleic acid denaturation, primer annealing and extension of the annealed primers by the action of a thermostable polymerase. A more detailed description as well as applications of PCR are provided in U.S. Pat. Nos. 4,683,195; 4,683,202; and 4,965,188; Saiki et al., 1985, Science 230:1350-1354; Mullis et al., 1986, Cold Springs Harbor Symp. Quant. Biol. 51:263-273; Mullis and Faloona, 1987, Methods Enzymol. 155:335-350; PCR Technology-principles and applications for DNA amplification, 1989, (ed. H. A.Erlich) Stockton Press, New York; PCR Protocols: A guide to methods and applications, 1990, (ed. M. A. Innis et al.) Academic Press, San Diego; and PCR Strategies, 1995, (ed. M. A. Innis et al.) Academic Press, San Diego, Barany, 1991, PCR Methods and Applic. 1:5-16); Gap-LCR (PCT Patent Publication No. WO 90/01069); each of which is incorporated by reference as if fully set forth herein.

In Allele-Specific PCR (also called the amplification refractory mutation system or ARMS) the assay occurs within the PCR reaction itself. Sequence-specific PCR primers which differ from each other at their terminal 3' nucleotide are used to only amplify the normal allele in one reaction, and only the mutant allele in another reaction. When the 3' end of a specific primer is fully matched, amplification occurs. When the 3' end of a specific primer is mismatched, amplification fails to occur.

Other amplification techniques include Ligase Chain Reaction, described in Wu and Wallace, 1989, Genomics 4:560-569 and Barany, 1991, Proc. Natl. Acad. Sci. USA 88:189-193,

incorporated by reference as if fully set forth herein; Strand Displacement Amplification; Nucleic Acid Sequence Base Amplification; Transcription Mediated Amplification; Repair Chain Reaction, described in European Patent Publication No. 439,182 A2), 3SR (Kwoh et al., 1989, Proc. Natl. Acad. Sci. USA 86:1173-1177; Guatelli et al., 1990, Proc. Natl. Acad. Sci. USA 87:1874-1878; PCT Patent Publication No. WO 92/0880A), and NASBA (U.S. Pat. No. 5,130,238), incorporated by reference as if fully set forth herein; Self-sustained Sequence Replication; Strand Displacement Amplification, etc., described in Manak, DNA Probes, 2nd Edition, p 255-291, Stockton Press (1993)), incorporated by reference as if fully set forth herein; and Non-Isotopic RNase Cleavage Assay, described in Goldrick MM, Kimball GR, Liu Q, Martin LA, Sommer SS, Tseng JYH. Nirca(Tm) - A Rapid Robust Method For Screening For Unknown Point Mutations. Biotechniques 1996;21:106-12, incorporated by reference as if fully set forth herein. Non-Isotopic RNase Cleavage Assay amplifies RNA. RNase enzymes, e.g., RNase 1 and RNase T1, increase the sensitivity of the assay.

Manufacturing Methods for a Sensor Array

As described above, after the cavities are formed in the supporting member, a particle may be positioned at the bottom of a cavity using a micromanipulator. This allows the location of a particular particle to be precisely controlled during the production of the array. The use of a micromanipulator may, however, be impractical for mass-production of sensor arrays. A number of methods for inserting particles that may be amenable to an industrial application have been devised.

In one embodiment, the use of a micromanipulator may be automated. Particles may be "picked and placed" using a robotic automated assembly. The robotic assembly may include one or more dispenser heads. A dispenser head may be configured to pick up and hold a particle. Alternatively, a dispenser head may be configured to hold a plurality of particles and dispensing only a portion of the held particles. An advantage of using a dispense head is that individual particles or small groups of particles may be placed at precise locations on the sensor array. A variety of different types of dispense heads may be used.

In one embodiment, a vacuum pick-up/dispense head may be used. The dispense head uses a vacuum system to pick up particles. The dispense head may be formed using small diameter tubing, with an inner diameter (ID) smaller than the particle outer diameter (OD). The dispense head may be coupled to a robotic control system via an arm. The robotic control system may be programmed to first move the dispense head to a storage location of the correct particle type, vacuum would be applied to the dispense head once it is "dipped" into the particle storage compartment, thus grasping one particle. The robotic control system would then move the arm such that the dispense head is in a position in close proximity to (or actual contact with) the appropriate location on the sensor array (See FIG. 70A). The dispense head vacuum would then be turned off (i.e., the vacuum would be removed), and if necessary a slight positive pressure could be applied to the dispense head. The particle would thus be dislodged from the dispense head onto the sensor array (See FIG. 70B).

The robotic control system may include a single dispense head or a plurality of dispense heads. The use of a plurality of dispense heads would allow multiple cavities of the sensor array to be filled during a single filing operation. In this manner the efficiency of filling the sensor array may be increased.

Another example of a robotic vacuum pick-up/dispense head is described in U.S. Patent No. 6,151,973 to Geysen which is incorporated herein by reference.

The dispense head could also be in the form of a "solid" pick-up wand. The solid dispense head may rely on natural attractive forces between a particle and the dispense head material to attach a particle to the dispense head. For example, when a particle is placed in close proximity to the dispense head, electrostatic interactions between the particle and the dispense head may cause the particle to "stick" to the dispense head. The dispense head may be placed at the appropriate location over a cavity of the sensor array (See FIG. 71A). When the particle is placed in close proximity to the sensor array, the attractive forces between the chip and particle, along with gravitational forces, may cause the particle to transfer from the dispense head to the sensor array (See FIG. 71B). For example, with PEG particles, a dispense head made of tungsten

will cause the PEG particle to attach to the tungsten tip, but the particle may still be transferred to a silicon based sensor array when brought into close proximity of the sensor array. A single solid dispense head or a plurality of solid dispense heads may be used.

In another embodiment, the dispense head could also be formed from one or more "pipettes" with an inner diameter greater than the diameter of the particles. Particles may be delivered directly into the bore of the pipette using a pump/dispense system. Such a system is similar to precision adhesive dispense systems in current use. The particles may be suspended in a liquid (e.g., water), and controlled amounts of the liquid would be pumped through the head to deliver a particle to the appropriate location on the sensor array chip. Such a dispensing system may have difficulties delivering only one particle at a time. Any extra particles, however, may be removed form the sensor array after application. Additionally, by making an array of pipettes the rate of particle placement may be increased. Other advantages of this approach may include the ability to deliver the particles in an aqueous environment if the particle chemistry so requires, as well making the deliver of different particles to each head fast and efficient, since no "pick up" step is required.

The "pipette" system relies on the use of controlled amounts of liquid to transport the particle from a storage area to the tip of the dispense head. In one embodiment, blast of air may be used to force a portion of the liquid toward the dispense head tip. In another embodiment, the dispense head may be made using technology essentially identical to that used in "ink-jet" printer heads. These heads typically rely on bursts of heat to quickly heat the liquid, causing bubbles of the liquid to be forced to the tip of the dispense head.

Once the pick-up/dispense head has delivered a particle or collection of particles to the appropriate location on the sensor array it may be desirable to insure that a single particle be collected at exactly the correct position on the sensor array. This may be accomplished using a vacuum chuck-like effect, as illustrated in FIGS. 72A-72D.

In one embodiment, the sensor array includes cavities used to locate and at least partially

contain the particles. When placed on a main vacuum chuck, each individual cavity may also acts as a vacuum chuck. The sensor array, when placed on a vacuum chuck may allow air-flow through the cavities. FIG. 72A depicts a multi-tip dispense head that allows the simultaneous application of many particles. The head is aligned to the cavities in the sensor array using an appropriate mechanical alignment system. If a particle is simply brought into proximity with the cavity, the fluid (e.g., air, but could also be a liquid) flow through the cavity may draw the particle into its proper location and hold it there (as depicted in FIG. 72B). In some embodiments, the dispense head may delivered more than one particle to a given cavity. Only one of the dispensed particles, however, is fully held in place by the vacuum at any give cavity. After the dispense head is moved away from the sensor array, excess particles may be removed using a side-directed jet (air or some other fluid) as depicted in FIG. 72C. The desired particles are held in their storage pits by the pressure differential across pits produced by the vacuum chuck. The process may now be repeated. In FIG. 72D another pipette head is illustrated that dispenses a distinct set of particles from that dispensed by the first head. This may allow more rapid dispensing of a larger variety of particle types.

When the sensor array is placed on a vacuum chuck, the particles may be picked up with a vacuum dispense head. The particles may then be pulled off of the dispense tool when the vacuum of the dispense head is released. The applied vacuum from the vacuum chuck may keep the particles from in the cavities. After the particles have been dispensed, a cover may be disposed on the sensor array to keep the particles in place. The cover may be attached to the sensor array using a pressure sensitive adhesive. After the cover is placed onto the sensor array, the vacuum may be released and the sensor array removed from the vacuum chuck.

Passive Transport of Fluid Samples

For some chemical sensor array systems, fluids may be transported into and across the sensor array during use. In one embodiment, fluids my be transferred into and through a sensor array using a system that relies on variations in the surface wetting characteristics of a channel. An advantage of such a system is that the system may be "passive" (i.e., no external power

source or components). Upon the introduction of a sample, the samples may be drawn into the system and distributed to the particles. This is particularly advantageous for small portable sensor array systems.

In one embodiment, a chemical sensor array is composed of a number of superimposed layers. FIG. 73 depicts a side-sectional view of the sensor array system. A support layer 1010 (e.g., a glass layer) is used as the foundation for the system. A spacer layer 1020 is formed upon the support layer. The support layer may be formed of a relatively inert material using standard semiconductor lithographic techniques. In one embodiment, the support layer may be formed from photoresist (e.g., a dry film photoresist). Alternatively, silicon nitride or silicon dioxide may be used as the spacer layer. The spacer layer may be patterned such that the spacer layer supports an outer portion of an overlying sensor layer 1030. This etching of the spacer layer 1020 may form a channel 1022 under the cavities formed in the sensor layer 1030. This channel 1022, may allow fluids to pass through the cavities and out of the sensor array system.

The sensor layer 1030 includes a number of cavities 1036 for holding a particle 1038. The formation of cavities in a sensor layer has been described earlier. In one embodiment, the sensor layer is formed from silicon. The silicon sensor layer may be partially etched such that an inlet and channel may be formed in the silicon layer. As depicted in FIG. 73 the outer portion of the sensor layer may be thicker than the interior portions. The application of a cover layer 1050, may be accomplished by resting the cover layer on the elevated portions of the sensor layer. This creates a channel 1042 between the cover layer and the sensor layer.

The etched portion of the sensor layer may be divided into segments coupled by a channel. FIG. 74A depicts a top view of the sensor array system and FIG. 74B depicts a bottom view. The first segment 1041 acts as a well or reservoir for the introduction of fluid samples. The second segment 1045 may include a number of cavities which include particles. The first segment may be coupled to the second segment by one or more channels 1043, formed in the sensor array. The channels allow the fluid to flow from the reservoir to the cavities. The cover

layer 1050 may be positioned over the support layer 1030 to form channel 1042. Materials and methods of for forming the cover layer have been described previously.

Referring back to FIG. 73, the conduction of a fluid through the channel may be accomplished using a combination of hydrophobic and hydrophilic surfaces. In one embodiment, a series of hydrophobic segments 1032 are applied to a surface of channels 1022 and 1042. A layer of a hydrophilic material 1034 may be placed on the opposite surface of the channel, with respect to the hydrophobic materials. When an aqueous fluid sample is introduced into the channel, the water is attracted toward the hydrophilic layer while being repelled by the hydrophobic layer. This attraction/repulsion creates a current within the channel. The hydrophilic surfaces may be composed of silicon or hexamethyldisilane. The hydrophobic surfaces may be composed of silicon dioxide, silicon nitride, silicon dinitride, siloxane, or silicon oxynitride.

The system depicted in FIG. 73 may cause a current to flow in a direction from the left side toward the right. Thus the fluid, introduced at inlet 1040, may flow through the channel 1042 in a direction toward the particles. After contacting the particles, the fluid may pass thorough the cavity and into the lower channel 1022. The hydrophilic and hydrophobic portions of the lower channel may induce a current that cause the fluid to flow toward the outlet of the sensor array system.

Likewise, the system depicted in FIG. 77 may cause current to flow in a direction from the left side to toward the right. Alternatively, the fluid may exit through the top portion of the system through the cover. The fluid may be introduced at inlet 1060 and may flow through channel 1062. Fluid may then flow through cavity 1064 past particle 1065. The fluid may also flow through cavity 1066 past particle 1067. The wall 1072 prevents the fluid from flowing past cavity 1066 in the in channel 1062. After flowing through the cavities, the fluid flows through channel 1068 and then up through cavity 1070. The hydrophilic and hydrophobic portions of the lower channel may induce a current that cause the fluid to flow toward the outlet 1074 of the

sensor array system. In addition, FIG. 73 depicts a bubble-trap 1035 that may consist of a wall in a hydrophobic region.

The sensor array may be formed from a plurality of layers. The layers may be assembled with dry film materials and ultraviolet curable epoxy. The support layer serves as a base for the system. The support layer may be formed of a variety of materials including, but not limited to glass, silicon nitride, silicon, silicon dioxide, plastic, and dry film photoresist. The support layer is depicted in FIG. 75D.

Onto the support layer is formed a spacer layer. The pattern for an embodiment of the spacer layer is depicted in FIG. 75C. The spacer layer may be placed in the locations that will not be directly under the cavities. The spacer layer may allow a channel to be formed under the sensor array.

The sensor layer is formed upon the spacer layer. A pattern for the etching of the sensor layer is depicted in FIG. 75B. The shaded areas 1031 represent the portion of the sensor layer that is etched to a thickness that is less than the remaining portion of the sensor layer 1033. The sensor layer may be formed from a variety of materials, including silicon, plastic, and dry film photoresist, as has been described before. The sensor layer may be aligned with the support layer to allow a channel to be formed under the cavities. The channel may allow fluids to pass from the sensor array system.

A cover layer is placed over the sensor layer. The etching of the cover layer may allow an upper channel 1042 to be formed between the sensor layer and the cover layer. The cover layer, in one embodiment, includes an opening 1052 that allows a fluid to be passed through the cover layer to the sensor layer. A pattern for the cover layer is depicted in FIG. 75A. The opening may be aligned with a reservoir section of the sensor layer.

In general, the use of a passive fluid transport system allows only a single use of the sensor array. Although the sensor array may have many chemical particles, and hence has multi-

analyte capability, the surface wetting "pump" may only be used once. For many testing situations (e.g., medical testing) this is not a significant problem, since it is desirable to dispose of the sensing element after a single use. If multiple testing of samples is to be performed an "array of arrays" may be used, as depicted in FIG. 76. In this case, multiple sample introduction sites, each coupled to its own suite of sensor sites, may be fabricated. This setup may allow multiple uses of the sensor array (i.e., use one sensor suite for each test) or allow the simultaneous analysis of multiple samples.

Portable Sensor Array System

A sensor array system becomes most powerful when the associated instrumentation may be delivered and utilized at the application site. That is, rather than remotely collecting the samples and bringing them to a centrally based analysis site, it may be advantageous to be able to conduct the analysis at the testing location. Such a system may be use, for example, for point of care medicine, on site monitoring of process control applications, military intelligence gathering devices, environmental monitoring, and food safety testing.

An embodiment of a portable sensor array system is depicted in FIG. 78. The portable sensor array system would, in one embodiment, have a size and weight that would allow the device to be easily carried by a person to a testing site. The portable sensor array system includes a light source, a sensor array, and a detector. The sensor array, in some embodiments, is formed of a supporting member which is configured to hold a variety of particles in an ordered array. The particles are, in some embodiments, elements which will create a detectable signal in the presence of an analyte. The particles may include a receptor molecule coupled to a polymeric bead. The receptors may be chosen for interacting with specific analytes. This interaction may take the form of a binding/association of the receptors with the analytes. The supporting member may be made of any material capable of supporting the particles. The supporting member may include a plurality of cavities. The cavities may be formed such that at least one particle is substantially contained within the cavity. The sensor array has been previously described in greater detail.

The portable sensor array system may be used for a variety of different testing. The flexibility of the sensor array system, with respect to the types of testing, may be achieved through the use of a sensor array cartridge. Turning to FIG. 78, a sensor array cartridge 1010 may be inserted into the portable sensor array system 1000 prior to testing. The type of sensor array cartridge used will depend on the type of testing to be performed. Each cartridge will include a sensor array which includes a plurality of chemically sensitive particles, each of the particles including receptors specific for the desired task. For example, a sensor array cartridge for use in medical testing for diabetes may include a number of particles that are sensitive to sugars. A sensor array for use in water testing, however, would include different particles, for example, particles specific for pH and/or metal ions.

The sensor array cartridge may be held in place in a manner analogous to a floppy disk of a computer. The sensor array cartridge may be inserted until it snaps into a holder disposed within the portable sensor system. The holder may inhibit the cartridge from falling out from the portable sensor system and place the sensor in an appropriate position to receive the fluid samples. The holder may also align the sensor array cartridge with the light source and the detector. A release mechanism may be incorporated into the holder that allows the cartridge to be released and ejected from the holder. Alternatively, the portable sensor array system may incorporate a mechanical system for automatically receiving and ejecting the cartridge in a manner analogous to a CD-ROM type system.

The analysis of simple analyte species like acids/bases, salts, metals, anions, hydrocarbon fuels, solvents may be repeated using highly reversible receptors. Chemical testing of these species may be repeatedly accomplished with the same sensor array cartridge. In some cases, the cartridge may require a flush with a cleaning solution to remove the traces from a previous test. Thus, replacement of cartridges for environmental usage may be required on an occasional basis (e.g., daily, weekly, or monthly) depending on the analyte and the frequency of testing

Alternatively, the sensor array may include highly specific receptors. Such receptors are particularly useful for medical testing, and testing for chemical and biological warfare agents. Once a positive signal is recorded with these sensor arrays, the sensor array cartridge may need to be replaced immediately. The use of a sensor array cartridge makes this replacement easy.

Fluid samples may be introduced into the system at ports 1020 and 1022 at the top of the unit. Two ports are shown, although more ports may be present. One 1022 may be for the introduction of liquids found in the environment and some bodily fluids (e.g., water, saliva, urine, etc.). The other port 1020 may be used for the delivery of human whole blood samples. The delivery of blood may be accomplished by the use of a pinprick to pierce the skin and a capillary tube to collect the blood sample. The port may be configured to accept either capillary tubes or syringes that include blood samples.

For the collection of environmental samples, a syringe 1030 may be used to collect the samples and transfer the samples to the input ports. The portable sensor array system may include a holder that allows the syringe to be coupled to the side of the portable sensor array system. One of the ports 1020 may include a standard luer lock adapter (either male or female) to allow samples collected by syringe to be directly introduced into the portable sensor array system from the syringe.

The input ports may also be used to introduce samples in a continuous manner. The introduction of samples in a continuous manner may be used, e.g., to evaluate water streams. An external pump may be used to introduce samples into the portable sensor array system in a continuous manner. Alternatively, internal pumps disposed within the portable sensor array system may be activated to pull a continuous stream of the fluid sample into the portable sensor array system. The ports are also configured to allow the introduction of gaseous samples.

In some cases it may be necessary to filter a sample prior to its introduction into the portable sensor array system. For example, environmental samples may be filtered to remove solid particles prior to their introduction into the portable sensor array system. Commercially

available nucleopore filters 1040 anchored at the top of the unit may be used for this purpose. In one embodiment, filters 1040 may have luer lock connections (either male or female) on both sides allowing them to be connected directly to an input port and a syringe.

In one embodiment, all of the necessary fluids required for the chemical/biochemical analyses are contained within the portable sensor array system. The fluids may be stored in one or more cartridges 1050. The cartridges 1050 may be removable from the portable sensor array system. Thus, when a cartridge 1050 is emptied of fluid, the cartridge may be replaced by a new cartridge or removed and refilled with fluid. The cartridges 1050 may also be removed and replaced with cartridges filled with different fluids when the sensor array cartridge is changed. Thus, the fluids may be customized for the specific tests being run. Fluid cartridges may be removable or may be formed as an integral part of the reader.

The fluid cartridges 1050 may include a variety of fluids for the analysis of samples. In one embodiment, each cartridge may include up to about 5 mL of fluid and be used for about 100 tests before being depleted. One or more of the cartridges 1050 may include a cleaning solution. The cleaning solution may be used to wash and/or recharge the sensor array prior to a new test. In one embodiment, the cleaning solution may be a buffer solution. Another cartridge 1050 may include visualization agents. Visualization agents may be used to create a detectable signal from the particles of the sensor array after the particles interact with the fluid sample. In one embodiment, visualization agents include dyes (visible or fluorescent) or molecules coupled to a dye, which interact with the particles to create a detectable signal. In an embodiment, a cartridge 1050 may be a vacuum reservoir. The vacuum reservoir may be used to draw fluids into the sensor array cartridge. The vacuum cartridge would act in an analogous manner to the vacutainer cartridges described previously. In another embodiment, a fluid cartridge may be used to collect fluid samples after they pass through the sensor array. The collected fluid samples may be disposed of in an appropriate manner after the testing is completed.

In one embodiment, an alpha-numeric display screen 1014 may be used to provide information relevant to the chemistry/biochemistry of the environment or blood samples. Also

included within the portable sensor array system is a data communication system. Such systems include data communication equipment for the transfer of numerical data, video data, and sound data. Transfer may be accomplished using either data or analog standards. The data may be transmitted using any transmission medium such as electrical wire, infrared, RF and/or fiber optic. In one embodiment, the data transfer system may include a wireless link (not shown) that may be used to transfer the digital chemistry/biochemistry data to a closely positioned communications package. In another embodiment, the data transfer system may include a floppy disk drive for recording the data and allowing the data to be transferred to a computer system. In another embodiment, the data transfer system may include a serial or parallel port connection hardware to allow transfer of data to a computer system.

The portable sensor array system may also include a global positioning system ("GPS"). The GPS may be used to track the area that a sample is collected from. After collecting sample data, the data may be fed to a server, which compiles the data along with GPS information. Subsequent analysis of this information may be used to generate a chemical/biochemical profile of an area. For example, tests of standing water sources in a large area may be used to determine the environmental distribution of pesticides or industrial pollutants.

Other devices may also be included in the portable sensor array that are specific for other applications. For example, for medical monitoring devices including but not limited to EKG monitors, blood pressure devices, pulse monitors, and temperature monitors.

The detection system may be implemented in a number of different ways such that all of the detection components fit within the casing of the portable sensor array system. For the optical detection/imaging device, either CMOS or CCD focal plane arrays may be used. The CMOS detector offers some advantages in terms of lower cost and power consumption, while the CCD detector offers the highest possible sensitivity. Depending on the illumination system (see below), either mono-chrome or color detectors may be used. A one-to-one transfer lens may be employed to project the image of the bead sensor array onto the focal plane of the detector. All fluidic components may be sealed away from contact with any optical or electronic components.

Sealing the fluids away from the detectors avoids complications that may arise from contamination or corrosion in systems that require direct exposure of electronic components to the fluids under test. Other detectors such as photodiodes, cameras, integrated detectors, photoelectric cells, interferometers, and photomultiplier tubes may be used.

The illumination system for colorimetric detection may be constructed in several manners. When using a monochrome focal plane array, a multi-color, but "discrete-wavelength-in-time" illumination system may be used. The simplest implementation may include several LED's (light emitting diodes) each operating at a different wavelength. Red, green, yellow, and blue wavelength LEDs are now commercially available for this purpose. By switching from one LED to the next, and collecting an image associated with each, colorimetric data may be collected.

It is also possible to use a color focal plane detector array. A color focal plane detector may allow the determination of colorimetric information after signal acquisition using image processing methods. In this case, a "white light" illuminator is used as the light source. "White light" LEDs may be used as the light source for a color focal plane detector. White light LEDs use a blue LED coated with a phosphor to produce a broad band optical source. The emission spectrum of such devices may be suitable for colorimetric data acquisition. A plurality of LEDs may be used. Alternatively a single LED may be used.

Other light sources that may be useful include electroluminescent sources, fluorescent light sources, incandescent light sources, laser lights sources, laser diodes, arc lamps, and discharge lamps. The system may also be configured to use external light source (both natural and unnatural) for illumination.

A lens may be positioned in front of the light source to allow the illumination area of the light source to be expanded. The lens may also allow the intensity of light reaching the sensor array to be controlled. For example the illumination of the sensor array may be made more uniform by the use of a lens. In one example, a single LED light may be used to illuminate the sensor array. Examples of lenses

that may be used in conjunction with an LED include Diffusing plate PN K43-717 Lens JML, PN61874 from Edmund scientific.

In addition to colorimetric signaling, chemical sensitizers may be used that produce a fluorescent response. The detection system may still be either monochrome (for the case where the specific fluorescence spectrum is not of interest, just the presence of a fluorescence signal) or color-based (that would allow analysis of the actual fluorescence spectrum). An appropriate excitation notch filter (in one embodiment, a long wavelength pass filter) may be placed in front of the detector array. The use of a fluorescent detection system may require an ultraviolet light source. Short wavelength LEDs (blue to near UV), may be used as the illumination system for a fluorescent based detection system.

In some embodiments, use of a light source may not be necessary. The particles may rely on the use of chemiluminescence, thermoluminescence or piezoluminescence to provide a signal. In the presence of an analyte of interest, the particle may be activated such that the paticles produce light. In the absence of an analyte, the particles may not exhibit produce minimal or no light.

The portable sensor array system may also include an electronic controller which controls the operation of the portable sensor array system. The electronic controller may also be capable of analyzing the data and determining the identity of the analytes present in a sample. While the electronic controller is described herein for use with the portable sensor array system, it should be understood that the electronic controller may be used with an of the previously described embodiments of an analyte detection system.

The controller may be configured to control the various operations of the portable sensor array. Some of the operations that may be controlled or measured by the controller include: (i) determining the type of sensor array present in the portable sensor array system; (ii) determining the type of light required for the analysis based on the sensor array ; (iii) determining the type of fluids required for the analysis, based on the sensor array present; (iv) collecting the data produced during the analysis of the fluid sample; (v) analyzing the data produced during the

analysis of the fluid sample; (vi) producing a list of the components present in the inputted fluid sample (vii) monitoring sampling conditions (e.g., temperature, time, density of fluid, turbidity analysis, lipemia, bilirubinemia, etc).

Additionally, the controller may provide system diagnostics and information to the operator of the apparatus. The controller may notify the user when routine maintenance is due or when a system error is detected. The controller may also manage an interlock system for safety and energy conservation purposes. For example, the controller may prevent the lamps from operating when the sensor array cartridge is not present.

The controller may also be configured to interact with the operator. The controller preferably includes an input device 1012 and a display screen 1014. A number of operations controlled by the controller, as described above, may be dependent on the input of the operator. The controller may prepare a sequence of instructions based on the type of analysis to be performed. The controller may send messages to the output screen to let the used know when to introduce samples for the test and when the analysis is complete. The controller may display the results of any analysis performed on the collected data on the output screen.

Many of the testing parameters may be dependent upon the type of sensor array used and the type of sample being collected. The controller will, in some embodiments, require the identity of the sensor array and test being performed in order to set up the appropriate analysis conditions. Information concerning the sample and the sensor array may be collected in a number of manners. In one embodiment, the sample and sensor array data may be directly inputted by the user to the controller. Alternatively, the portable sensor array may include a reading device which determines the type of sensor cartridge being used once the cartridge is inserted. In one embodiment, the reading device may be a bar code reader which is configured to read a bar code placed on the sensor array. In this manner the controller can determine the identity of the sensor array without any input from the user. In another embodiment, the reading device may be mechanical in nature. Protrusions or indentation formed on the surface of the sensor array cartridge may act as a code for a mechanical reading device. The information

collected by the mechanical reading device may be used to identify the sensor array cartridge. Other devices may be used to accomplish the same function as the bar code reader. These devices include, but are not limited to, smartcard readers and RFID systems.

The controller may also accept information from the user regarding the type of test being performed. The controller may compare the type of test being performed with the type of sensor array present in the portable sensor array system. If an inappropriate sensor array cartridge is present, an error message may be displayed and the portable sensor array system may be disabled until the proper cartridge is inserted. In this manner, incorrect testing resulting from the use of the wrong sensor cartridge may be avoided.

The controller may also monitor the sensor array cartridge and determine if the sensor array cartridge is functioning properly. The controller may run a quick analysis of the sensor array to determine if the sensor array has been used and if any analytes are still present on the sensor array. If analytes are detected, the controller may initiate a cleaning sequence, where a cleaning solution is passed over the sensor array until no more analytes are detected. Alternatively, the controller may signal the user to replace the cartridge before testing is initiated.

Another embodiment of a portable sensor array system is depicted in FIGS. 79A and 79B. The portable sensor array 1100 includes a body 1110 that holds the various components used with the sensor array system. A sensor array, such as the sensor arrays described herein, may be placed in cartridge 1120. Cartridge 1120 may support the sensor array and allow the proper positioning if the sensor array within the portable sensor system.

A schematic cross-sectional view of the body of the portable sensor array system is depicted in FIG. 79B. The cartridge 1120, in which the sensor array is disposed, extends into the body 1110. Within the body, a light source 1130 and a detector 1140 are positioned proximate to the cartridge 1120. When the cartridge 1120 is inserted into the reader, the cartridge may be held, by the body 110, at a position proximate to the location of the sensor array within the cartridge. The light source 1130and detector1140 may be used analyze samples disposed within

the cartridge. An electronic controller 1150 may be coupled to detector. The electronic controller 1150 may be configured to receive data collected by the portable sensor array system. The electronic controller may also be used to transmit data collected to a computer.

An embodiment of a cartridge for use in a sensor array system is depicted in FIG. 80. The cartridge include a carrier body 1210,.that is formed of a material that is substantially transparent to a wavelength of light used by the detector. IN one embodiment, plastic materials may be used. Examples of plastic materials that may be used include polycarbonates and polyacrylates. In one embodiment the body may be formed from Cyrolon AR2 Abrasion Resistant polycarbonate sheet at thicknesses of 0.118 inches and 0.236 inches. A sensor array gasket 1220 may be placed on the carrier body 120. The sensor array gasket 1220, may help reduce or inhibit the amount of fluids leaking from the sensor array. Leaking fluids may interfere with the testing being performed.

A sensor array 1230 may be placed onto the sensor array gasket. The sensor array may include one or more cavities, each of which includes one or more particles disposed within the cavities. The particles may react with an analyte present in a fluid to produce a detectable signal. Any of the sensor arrays described herein may be used in conjunction with the portable reader.

A second gasket 1240, may be positioned on the sensor array. The second gasket 1240, may be disposed between the sensor array 1230 and a window 1250. The second gasket 1240 may form a seal inhibiting leakage of the fluid from the sensor array. The window may be disposed above the gasket to inhibit damage to the sensor array.

The assembly may be completed by coupling a cover 1270 to the body 1210. A rubber spring 1260 may be disposed between the cover and the window to reduce pressure exerted by the cover on the window. The cover may seal the sensor array, gaskets, and window into the cartridge. The sensor array, gaskets and window may all be sealed together using a pressure sensitive adhesive. Examples of a pressure sensitive adhesive include Optimount 237 made by Seal products. Gaskets may be made from polymeric materials. In one example, Calon II - High

Performance material from Arlon may be used. The rubber spring may be made form a silicon rubber material.

The cover may be removable or sealed. When a removable cover is used the cartridge may be reused by removing the cover and replacing the sensor array. Alternatively, the cartridge may be a one use cartridge in which the sensor array is sealed within the cartridge.

The cartridge may also include a reservoir 1270. The reservoir may be configured to hold the analyte containing fluid after the fluids pass through the sensor array. FIG. 81 depicts a cut away view of the cartridge that shows the positions of channels formed in the cartridge. The channels may allow the fluids to be introduced into the cartridge. The channels also may conduct the fluids from the inlet to the sensor array and to the reservoir.

In one embodiment, the cartridge body 1210, includes a number of channels disposed throughout the body. An inlet port 1282 is configured to receive a fluid delivery device for the introduction of fluid samples into the cartridge. In one embodiment, the inlet port may include a luer lock adapter, configured to couple with a corresponding luer lock adapter on the fluid delivery device. For example, a syringe may be used as the fluid delivery device. The luer lock fitting on the syringe may be coupled with a mating luer lock fitting on the inlet port 1282. Luer lock adapters may also be coupled to tubing, so that fluid delivery may be accomplished by the introduction of fluids through appropriate tubing to the cartridge.

The introduced fluid passes through channel 1284 to channel outlet 1285. Channel outlet 1285 may be coupled to an inlet port on a sensor array (see description of sensor arrays herein). Channel outlet 1285 is also depicted on FIG. 80. The fluids travels into the sensor array and through the cavities. After passing through the cavities, the fluid exits the sensor array and enters channel 1286 via channel inlet 1287. The fluid passes through channel 1286 to reservoir 1280. To facilitate the transfer of fluids through the cartridge, the reservoir may include an air outlet port 1288. Air outlet port 1288 may be configured to allow air to pass out of the reservoir, while retaining any fluids disposed within the reservoir. In one embodiment, the air outlet port 1288

may be an opening formed in the reservoir that is covered by a semipermeable membrane. A commercially available air outlet port includes a DURAVENT container vent, available from W.L. Gore. It should be understood, however, that any other material that allows air to pass out of the reservoir, while retaining fluids in the reservoir may be used. After extended use the reservoir 1280 may become filled with fluids. An outlet channel 1290 may also be formed extending through the body 1210 to allow removal of fluids from the body. Fluid cartridges 1292 for introducing additional fluids into the sensor array may be incorporated into the cartridges.

Magnetic Particle Production and Use

Magnetic particles may be made by different methods. In an embodiment, a solution containing Fe(II) and Fe(III) (typically $FeCl_2$ and $FeCl_3$), and a polymer (e.g. a protein) having available coordination sites may be treated (by titration or otherwise) with a strong base such as aqueous ammonia in order to precipitate magnetic iron oxides such as magnetite (Fe_3O_4) in a form which is intimately combined with the polymer. The precipitation may be typically carried out with rapid stirring and optional agitation by sonication, in order to produce resuspendable magnetic-polymer particles.

After precipitation, the particles may be washed and subsequently resuspended in a buffer solution at approximately neutral pH. Other embodiments may involve the use of metals other than iron in the coprecipitation reaction. In particular, Fe(III) may be replaced by any of a wide range of transition metal ions. In some cases, iron may be completely supplanted by appropriately selected transition metal ions. In some cases, the use of metals other than iron produces colored particles ranging from white to dark brown.

Magnetic-polymer particles may be produced of varying size. Magnetic particles may be tailor-made to include specific biofunctional ligands useful in various analytical, diagnostic, and other biological/medical applications. Magnetic particles may be produced with select chemical reagents that may be useful in various analytical applications.

Subsequent to precipitation and resuspension of the magnetic-polymer particles, they may be treated with a bifunctional reagent in order to cross-link reactive sites present on the polymer. This cross-linking may be effective as either an intra-particulate cross-linking in which reactive sites are bound on the same particle, or may be a reaction of an extra-particulate ligand which may then be cross-linked to the polymer on a given particle. In the second case, a bifunctional reagent having a relatively short distance between its two functional groupings may be desirable to promote linkage between the particle polymer and the extra-particulate species. Conversely, intra-particulate cross-linking may be promoted by the use of a bifunctional reagent which may be longer and may not be sterically hindered from bending so that two reactive sites on a single particle may be linked by a single bifunctional molecule.

As an alternative to the use of sonication during either the precipitation or resuspension steps outlined above, another type of agitation (such as mechanical stirring) may be employed.

Resuspension of the magnetic-polymer particles may be typically carried out in a low ionic strength buffer system (e.g. 40 mM phosphate). The buffer system may enable resuspension of particles which are not resuspendable in non-ionic solutions. In addition to phosphate buffers, borate and sulfate systems may also be used. The association of polymer and metal may result from coordination of metal present during coprecipitation by coordination sites on the polymer. It may be that certain coordination sites are more "available" than others, based on both the strength of the coordinate bond which may be formed by the particular atom, and the spatial hindrances imposed by surrounding atoms. It is known, for instance, that oxygen atoms having a "free" electron pair complex iron more strongly than amine nitrogen atoms and, to an even greater degree, a hydroxyl oxygen atom. Thus, a polymer bearing oxy-acid functional groups may provide better product particles than an amine-substituted polymer. Similarly, coordination sites which may be freely approached to close distances may yield better performance than sites which are hindered in either a path of approach or in approach distance.

The above-described trends may be qualitatively observable in various experiments. The

presence of "available coordination sites" appears necessary to the production of the resuspendable magnetic-polymer particles. For example, such diverse polymers as natural proteins, synthetic proteins, poly-amino acids, carboxy-poly-alkyls, alkoxy-poly-alkyls, amino-poly-alkyls, hydroxy-poly-alkyls, and various copolymers of these have all been demonstrated to produce suitable particles. In addition, other polymers such as sulfoxy-poly-alkyls, poly-acrylamines, poly-acrylic acid, and substituted poly-alkylenes may produce similar particles.

In selecting the transition metals to be employed in the coprecipitation reaction, several criteria may be important. First, the final compound must have one or more unpaired electrons in its structure. Second, one of the metals must possess an available coordination site for bonding to a polymer. Third, the coprecipitate must be capable of forming a cubic close-packed or hexagonal close-packed (eg. for cubic: spinel or inverse spinel) crystalline structure. This last requirement may be due to the need for a very close packing in order for a compound to be magnetic.

In an embodiment, polymers useful in preparing the magnetic particles may be "tailor-made" to include monomers which may exhibit a specific biofunctional activity. Using such a polymer may permit direct precipitation of a biofunctional magnetic-polymer particle which may require little or no further treatment in order to be useful in assays which rely on the particular biofunctional activity of the polymer.

In some embodiments, larger, less stable particles may be useful. The particles may be made to agglomerate while still retaining both their biofunctional and magnetic characteristics. Agglomeration of the particles may be accomplished by treatment of a suspension with a predetermined amount of, for example, barium chloride solution. This treatment may be designed to cause the particles to settle out of suspension in a predetermined period of time in order to allow the performance of further procedures, or to allow the larger particles to be easily attracted by relatively small magnets. U.S. Patent No. 4,795,698 to Owen et al., which is incorporated herein by reference, provides further details for producing magnetic particles.

Magnetic particles may also be produced from metallocenes and metal hydroxide compounds. These particles may then be incorporated into polymeric materials to produce magnetically active particles.

Metallocenes are cycopentadienyl coordinate complexes of metals. The cyclopentadienyl group, C_5H_5, has long been known to form complexes with metals or metalloidal atoms. In an embodiment, metallocenes may be cyclopentadienyl complexes of transition metals. The transition-metals may include, for example, iron (Fe), magnesium (Mg), manganese (Mn), cobalt (Co), nickel (Ni), zinc (Zn) and copper (Cu). Particularly useful metallocenes may be ferrocene $(C_5H_5)_2Fe$, nickelocene, $(C_5H_5)_2Ni$, and cobaltocene, $(C_5H_5)_2Co$. Metallocenes have the general formula $(C_5H_5)_2$ M, wherein M is the metal and have a "sandwich" configuration. The structure of metallocenes endows these molecules with high thermal stability (e.g., up to about 500.°C. for ferrocene).

In an embodiment, an aqueous slurry of the metallocene may be produced. The slurry may be prepared, for example, by combining the metallocene compound and water, and mixing or by milling in a high energy mill, such as a sand mill or a ball mill. The length of time for which the slurries are milled will depend upon the particle size of the product which may be desired. The slurry may generally contain from about 0.1 to about 40 percent (%) by weight of the metallocene. A slurry containing from about 20 to about 25% by weight metallocene may be particularly useful.

The aqueous metallocene slurry may be combined with a second aqueous slurry of a metal hydroxide. The choice of metal hydroxide may depend upon the properties of the particles which may be desired. For example, to produce magnetite particles, iron (II) hydroxide (ferrous hydroxide) may be used. Other metal hydroxides which may be used to produce magnetic particles may include cobalt (II) hydroxide, cobalt (III) hydroxide, iron (III) hydroxide and nickel hydroxide. Slurries of these metal hydroxides may be prepared by precipitating a salt of the metal (e.g. chloride or sulfate salt) in an aqueous medium using a base, such as sodium hydroxide or ammonium hydroxide. An aqueous iron (II) hydroxide slurry may be prepared by precipitating

an aqueous solution of ferrous chloride or ferrous sulfate with ammonium or sodium hydroxide to form ferrous hydroxide (FeO(OH)). The resulting gelatinous precipitate of iron (II) hydroxide may be filtered, and the solid material may be collected, combined with water and milled in a high energy mill to form the slurry. The metal hydroxide slurry may contain from about 0.1 to about 40 percent (%) by weight of the metal hydroxide.

The two slurries may be combined and the mixture may be milled in a high energy mill, such as a commercial ball or sand mill, for a period of time sufficient to form fine magnetic particles, generally for about 1 hour to about 60 hours. Generally, the longer the milling step, the smaller the particles which may be formed.

In an embodiment, magnetite particles may be formed from iron (II) hydroxide and ferrocene according to the following equation:

$$2FeO(OH) + Fe(C_5H_5)_2 \rightarrow Fe_3O_4 + 2(C_5H_5) + H_2O + H_2 \text{ (gas)}$$

The iron (II) hydroxide powder may be milled in intimate contact with the ferrocene. Over a period of about 20 to 40 hours, the two materials may react by slow dissociation of the hydroxide to form magnetite, free cyclopentene, water and hydrogen. It may be necessary to allow sufficient void space in the mill, or to vent the mill periodically to accommodate the release of the hydrogen gas formed during the reaction. The particles may then be isolated and incorporated into polymeric materials to produce beads comprising magnetic particles. Additional production details may be found in U.S. Patent No. 5,071,076 to Chagnon et al., which is incorporated herein by reference.

In an embodiment, colloidal polymer or protein magnetite may be prepared with highly controllable, polymer/protein magnetite ratios. Typically, the particles may be precipitated from solutions of hydrated ferric and ferrous chlorides at 3.5 and 1.5 mg/ml, respectively, with protein content ranging from 500 ug/ml to 1.5 mg/ml. After appropriate washing, resuspension and sonication of such precipitates, colloidal, magnetically responsive particles may be produced,

wherein the mean diameter of particles may be approximately inversely proportional to starting protein concentrations. Particles about 20 nanometers or less in diameter may be obtained at the higher protein concentrations, whereas particles approximately 100 nanometers in diameter may be obtained at the lower end of the range of protein concentrations. It has been found that the ease with which various of these colloidal solutions may be salted out may be inversely related to the protein concentration of the solution and may be directly related to particle size. In other words, the smaller, higher protein containing particles may be more difficult to salt out. These results suggest that the particles having higher protein concentration may be more lyophilic, which might be expected because of the greater interaction between solvent water and protein, as compared with magnetite. Other possible explanations for this observed phenomenon may be that the magnetic cores of the larger colloidal particles may be easier to flocculate because of their magnetic moments, or that the smaller particles offer relatively larger surface area and consequently more surface charge to be neutralized.

In an embodiment, colloidal, magnetically responsive particles bearing (i) a biospecific binding material having binding affinity for the target substance of interest or (ii) a suitable retrieval agent, for example, anti-fluorescein, where a fluoresceinated receptor for the target substance may be used, may be incubated with an appropriately labeled specific binding substance and test sample suspected of containing the target substance, under conditions such that agglomeration of such particles may not occur. Agglomeration may not occur, for instance, because the binding capacity of the specific binding substance or the concentration of the target substance in the test medium may be too low. Following the binding of sufficient labeled substance (or inhibition thereof), an agglomerating agent, which may be either non-specific, or specific, preferably the former, e.g., a simple salt solution, may be added to the incubation mixture to cause agglomeration. Agglomeration may be brought about by the addition of a second non-specific agglomerating agent, e.g., an appropriately chosen colloid, if desired.

Alternatively, agglomeration may be effected by means of a specific agglomerating agent capable of cross-linking a component of the colloidal magnetic particles, such as a specific antibody. The resulting agglomerate may be removed from solution via centrifugation, filtration

or, via magnetic separation. It may also be possible to use a non-specific and/or specific agglomerating agents in various combinations, if desired. Thus, second colloid addition plus salting out may be feasible, as may the use of a second magnetically responsive colloidal particle bearing a receptor capable of cross-linking with a substance present on the colloidal protein magnetite initially added to the test sample.

Another useful application of the conversion of colloidal material to a magnetically separable form by the addition of a second colloid, may be to use protein colloidal magnetite as the agglomerating agent for some other non-magnetic colloidal material, where the latter bears the target substance of interest.

Colloidal reagents and non-specific or specific agglomerating agents may be added to the test medium simultaneously, rather than sequentially, as previously described. This may be accomplished by adding a suitable agglomerating agent to one of the colloidal reagents used in the assay, so that conversion of the colloid takes place after a substantial level of ligand/receptor interaction has occurred. Further information on production of magnetic colloidal particles may be found in U.S. Patent No. 5,108,933 to Liberti et al., which is incorporated herein by reference.

In an embodiment, permanently magnetized materials may be used to produce magnetic particles. Previously discussed agglomeration techniques may be used to form particles in which the particle composition may encapsulate the magnetic material. In an embodiment, the magnetic material may be suspended in a solution from which the particles may be formed. As the particles begin to form, due to agglomeration or other methods, the suspended magnetic material may be encapsulated thereby forming a magnetic particle. Magnetic material may also be incorporated into particles by physical means. In an embodiment, magnetic materials may be intermixed with particles using methods such as, but not limited to ball mills, low intensity mixers, and pug mills. A wide variety of magnetized materials may be used in the magnetic particles. Examples of magnetized materials, besides those materials previously discussed, may include, but are not limited to materials such as alnico, ferrite, barium ferrite, strontium ferrite, neodymium iron boron, samarium cobalt, iron oxide, or other ferromagnetic materials.

Upon formation of the magnetic particle, the magnetic particle may be further modified with target analyte materials. Eventually, the magnetic particles may be placed within the sensor array. In an embodiment, the magnetic particles may be located within the cavity or cavities of a sensor array by placement of permanent magnets in such a manner that the magnetic particle may be directed to a particular location, in this instance, a cavity in the sensor array. In an embodiment, a permanent magnet may be located under a cavity of interest. A solution containing suspended magnetic particles may be allowed to flow over the cavity, wherein a magnetic particle may be directed into the cavity by the interaction of the magnetic particle and the permanent magnet. Depending upon the cavity size, other particles may or may not be directed into the cavity. For example, a cavity only large enough to include one magnetic particle, may capture one particle, but, based upon space limitation, no further particles may be directed into the cavity. Conversely, a cavity large enough to include several particles may have several particles directed toward it before the cavity may no longer capture particles. When the desired cavity or cavities may be filled, a cover layer may be added to the substrate to retain the particles as discussed in previous sections. Directing magnetic particles to magnets for collection or to a particular location are further discussed in U.S. Patent No. 4,813,277 to Miller, et al, which is incorporated herein by reference.

Permanent magnets may be used to direct magnetic particles into cavities, but other embodiments may be possible. In an embodiment, electromagnets may be located at a desired cavity, such that the magnetic particle may be drawn into the desired cavity. For example, a flow of magnetic particles may be allowed to pass over the sensor array. An electromagnet may be located under a cavity such that as energy may be supplied to the electromagnet, a flowing magnetic particle may be directed into the desired cavity. A plurality of cavities may be located on the sensor array and a discrete electromagnet may be assigned to each cavity. Current flow to each electromagnet may be monitored such that a magnetic particle or particles may be directed to individual cavities. By controlling the electrical current to the electromagnets, some cavities may be filled with magnetic particles while other cavities may remain empty. A second flow of different magnetic particles may be allowed to flow over the sensor array, at which time other

electromagnets may be activated thereby causing the different magnetic particles to be directed into the currently empty cavities. This procedure may continue using other different magnetic particles until the selected cavities may be filled. In this way, various cavities may be filled with different magnetic particles. Other embodiments may allow location of multiple magnetic particles within the same cavity thereby providing the ability to detect multiple analytes from the same cavity. Other variations of cavities and particles may be possible wherein the variations may not be limited by the foregoing embodiments. U.S. Patent No. 5,981,297 to Baselt, which is incorporated herein by reference, further describes the recognition of magnetic particles with magnets

Formation of Cavities With Retaining Projections

In an embodiment, a mask may be deposited on a bulk crystalline (100) silicon substrate to form an integrated cover layer. The cover layer may be, but is not limited to, silicon nitride, a plastic, silicon dioxide, or a dry film photoresist material. The cover layer may be formed or etched in such a way that, after etching of the silicon substrate, various flexible micromachined projections may be present in the cover layer. Many types of structures formed on the cover layer may provide for development of flexible projections after etching. Examples of structures that may be formed on the cover layer may be, but are not limited to: star; cross; circle; square; or any other type of formation that provides for flexible projections after etching. In an embodiment, a cross, formed by an equal length upright with a transverse beam may be formed in the cover layer. An amount of the cover layer may be removed such that the substrate may be exposed to an etchant material. After removing the desired amount of cover layer, the substrate may be etched anisotropically. The etchant may continue to remove the silicon substrate until the bounding (111) planes may be reached. The resulting cavity may be a pyramidal shape into the silicon substrate. The pyramidal shaped cavity may enhance fluid flow. The cavity may be formed such that a bottom opening may also be present.

The flexible projections formed from the undercutting of the silicon substrate beneath the cover layer may provide a method of retention of the particle. In an embodiment, the flexible

projections may be produced from a mask opening which may be smaller than the underlying cavity. The particle may then be manipulated past the flexible projections into the cavity. As the particle passes the flexible projections, the flexible projections may be displaced downward until the particle passes completely by the flexible projections and into the cavity. As the particle passes the flexible projections, the flexible projections may return to their original position, thereby providing retention of the particle in the cavity. Retention of the particle in the cavity may be maintained by the flexible projections during subsequent handling of the sensor array.

Figure 82 shows the placement of a particle (1) into a cavity. The particle may be placed proximate to the cavity on top of the flexible projections (2) as shown in (a), at which time a micromanipulator may be used to press the particle past the flexible projections. The flexible projections may bend as the particle may be pressed past the projections, as shown in (b) and (c), until the particle may be placed within the cavity. The flexible projections may return to their normal position, as shown in (d), as the particle moves past the flexible projections and is substantially retained within the cavity.

The flexible projections may provide for specific size selection of particles to be placed into the cavity. In an embodiment, it may be assumed the particles may have a gaussian distribution. In a non-limiting example, an opening may be provided in the cover layer by the flexible projections which may be an opening larger than the mean size of the particle times a sigma value. The sigma value as defined hereinafter is the variability in size of a particle around a mean particle diameter of a gaussian distribution of particles. The bottom opening of the cavity may be an opening smaller than the mean size of the particle times a sigma value. If a 10% sigma value of the particle diameters may be assumed and a 10% sigma value of the top and bottom cavity openings, only the next size up or down may have a chance of filling the cavity. Assuming these variables, the probability for placing a particle the next size up in the cavity may be about one part in one thousand. The probability of placing a particle the next size down in the cavity may be about 1 in 300. Reduction in the variability of the particle size and reduction in the variability in the top and bottom openings of the cavity may result in a higher percentage of correctly sized particles being placed in the cavity.

In an embodiment, another strategy which may be employed with bead capture selectivity probability may be the use of three cavities of a desired size to provide triple redundancy. In this strategy, three cavities of the selected size may be used and selection criteria designed such that if two cavities contain the correct particle size, the cavities may be considered correctly filled. An error may result if two-same sized cavities may be incorrectly filled. The foregoing criteria may provide for a selection of the probability of placing a too large particle of about 1 in 10^6 and placement of a too small particle of about 1 in 77, 000. Error rates may be further reduced by decreasing the variability of the particle diameter and variability in the cavity top and bottom openings.

In an embodiment, a particle may be placed in a cavity using various techniques such as individual placement of particles. Micromanipulators may be used in the individual placement of a particle. A vacuum or flow system may be used to provide for more rapid production of cavity arrays compared to individual placement of particles into cavities. In an embodiment, a wafer may be fabricated with the correct top and bottom cavity openings to select the desired particle size. A solution of particles with a wide range of size distribution may be produced. The wafer may then be dipped into the solution whereupon a vacuum or flow may pull the particles past the cavity top flexible projections. A too large particle may not pass the top opening and a too small particle may pass through the cavity and out the cavity bottom. A correctly sized particle may pass the top opening flexible projections and be retained on the cavity bottom. In the embodiment, the flexible projections may be used as cavity opening discriminators. Flexing of the projections as the particle passes may not be necessary.

A combination of correctly sized flexible projections and particles may be used to produce a backflow preventer and pump. In an embodiment, a cavity may be fabricated such that the slits in the cover layer produce a rectangular bottom opening. The top layer may be fabricated such that a round opening slightly smaller than the particle may be produced. The flexible projections of the bottom opening may be designed for placement of a particle into the cavity. The fluid flow may be inhibited or stopped if the flow direction forces the particle against

the round top opening. If the flow is reversed, the particle may be forced against the flexible projections and depending upon the design of the flexible projections, flow may occur or may be significantly inhibited. For example, the flexible projections may be designed such that the slits may be as small as possible resulting in a significant decrease in back-flow capabilities. The effect of this embodiment may produce a valve with a high flow coefficient for flow in one direction and a low flow coefficient in the opposite direction.

The flexible projections may be designed to bend in one direction more favorably than in the opposite direction. In an embodiment, multiple lithography or deposition steps for producing the cover layer may provide a flexible projection which may flex preferably in the direction to allow placement of a particle within the cavity. The flexibility may be reduced in the direction in which the projections may be required to flex for removal of the particle. Providing enhanced flexibility in only one flexural direction may allow reduction of slit size in the cover layer needed to provide etch access to the silicon substrate.

In an embodiment, the flexible projections may be produced by undercutting the silicon substrate as described previously. The top cover flexible projections and bottom cover opening may be fabricated to the diameter desired, such that a particle may only be accepted in a shrunken state. The particle to be placed within the cavity may be exposed to a medium in which the particle may be caused to shrink. The shrunken particle may then be placed within the cavity at which point the particle may be exposed to a medium which causes the particle to return to it's normal diameter state. The particle may then be captured within the cavity. Correctly designing the swollen state of the particle and the flexible projections, the particle may be retained within the cavity subsequent to further processing of the array.

The sensor array may be used as a method for sorting various sized particles. In an embodiment, the sensor array may be fabricated with various sized cavities which may capture various sized particles. Depending upon etch time, the cavity sizes may be configured to different sizes. A shorter etch time may produce a smaller cavity size based upon the depth of the cavity into the substrate.

In an embodiment to provide selection of only one particle size from a distribution of particle sizes, a solution of particles with a wide range of particle size distribution may be allowed to flow over the substrate. A vacuum or flow may be used to pull the particles past the cavities etched into the substrate support. Those particles with a too large diameter may not be captured by a cavity where the top opening may be smaller than the particle diameter. The too large particle may continue to flow across the sensor array. Those particles with a smaller diameter than the bottom opening may be drawn into the cavity as they pass the top opening, but the small diameter particle may pass through the bottom opening and out of the substrate support. Particle sizes smaller than the top opening, but larger than the bottom opening may be drawn into the cavity and retained within the cavity. Those particles larger than the top opening and smaller than the bottom opening may not be retained on the substrate support. The non-retained particles may flow away from the substrate support. The flow may then be stopped and the substrate along with the captured particles may be removed from the flow. A reverse flow may then be used to dislodge the particles into a desired location.

In an embodiment, the array may provide an ability to pick and place many particles at once. The substrate may be fabricated with top and bottom openings designed to select a certain desired particle size. A solution of particles may be flowed over the substrate. Those particles of the desired particle size may be captured by the cavities as discussed in the previous section. The flow may then be stopped and the substrate, along with the captured particles, may be removed from the flow. A reverse flow may then be used to dislodge the particles into a desired location.

Further modifications and alternative embodiments of various aspects of the invention will be apparent to those skilled in the art in view of this description. Accordingly, this description is to be construed as illustrative only and is for the purpose of teaching those skilled in the art the general manner of carrying out the invention. It is to be understood that the forms of the invention shown and described herein are to be taken as the presently preferred embodiments. Elements and materials may be substituted for those illustrated and described herein, parts and processes may be reversed, and certain features of the invention may be utilized independently, all as would be apparent to one skilled in the art after having the benefit of this

description of the invention. Changes may be made in the elements described herein without departing from the spirit and scope of the invention as described in the following claims.